REMARKS

Claims 1-16, 19-32, 34 and 35 currently appear in this application. The Office Action of April 13, 2007, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicant respectfully requests favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

Restriction/Election

It is noted that the restriction requirement has been maintained, and that claims 1-25 have been withdrawn from consideration.

Rejections Under 35 U.S.C. 112

Claims 26-35 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

This rejection is respectfully traversed.

Claims 26-35 have been amended to delete "or substantially free of other amino acids."

Art Rejections

Claims 26-34 are rejected under 35 U.S.C. 102(b) as being anticipated by Kleinberger et al., US 4,259,393.

This rejection is respectfully traversed.

Kleinberger teaches treating hepatic encephalopathy by administering L-valine, which the Examiner argues would inherently treat a patient suffering forma low albumin level. As the present specification notes at page 2, lines 61-21, hepatic encephalopathy is one of the complications of worsened hepatic diseases and toxic substances such as ammonia that accumulate in blood impair the central nervous system to cause various neurotic symptoms; hence, hepatic encephalopathy is different from "hepatic disease" as defined in the present application.

Hepatic encephalopathy is one of the disorders caused by impaired hepatic function, and encompasses a variety of symptoms such as manic-depressive condition, and deep coma. Thus, hepatic encephalopathy is diagnosed only when taking into account states such as a psychiatric state, degree of hepatic malfunction, abnormal electroencephalogram, and severity of hyperammonia. However, it is currently understood in a clinical environment that the severity of hepatic malfunction is not well correlated with the severity of hepatic encephalopathy. This means that a method of treating

hepatic encephalopathy is not necessarily applicable to treat hepatic malfunction, even more to improve a low albumin level in a patient.

Further, although Kleinberger provides a vague statement that high dose administration of L-valine advantageously influences hepatic encephalopathy, Kleinberger has no concrete data regarding the efficiency of L-valine. Kleinberger asserts that administration of L-valine diminishes ammonia in tissue (column 1, lines 57-60). Moreover, Kleinberger does not teach or suggest that L-valine has a beneficial effect on hypoalbuminemia there is nothing in Kleinberger that teaches or suggests that L-valine has a beneficial effect on hypoalbuminemia.

Additionally, referring to column 1, lines 57-58 of Kleinberger, one skilled in the art would understand form this reference is only that high dose administration of L-valine results in reducing the ammonia levels in tissue, thereby improving hepatic encephalopathy. One skilled in the art would not extract from Kleinberger the concept that L-valine can be used to improve low albumin level, since there is no relationship between reducing ammonia in tissue and improving the albumin level.

Claims 26-35 are rejected under 35 U.S.C. 102(a) as being anticipated by Ichihara et al. (WO 96/00059) or under

35 U.S.C. 102(e) as being anticipated by Nishihira et al., US 5,916,921, the English equivalent of WO 96/00059,

This rejection is respectfully traversed.

The Examiner asserts that Nishihira inherently discloses the feature of the present application, such as "improving a low albumin level" and "improving hypoalbuminemia." However, as will be explained below, it is respectfully submitted that treating hepatic disease in Nishihara is different from the treatment or improvement or patients exhibiting a low albumin level.

Submitted herewith is a copy of Sekas et al., *J. Exp. Path.* (1979) **60**:447-452 in which hepatectomized rats were used as in Nishihara. At page 449, right column, lines 17-13 from the bottom of the column, the authors noted that "Serum albumen concentration was fairly constant… over the 2 weeks of the study." This shows that the remaining 30% of liver in the rat retains a function sufficient for maintaining a balance between production and metabolism of albumin. This is consistent with the case of human liver, where it is known that a normal liver can be 70% hepatectomized without adverse effect on liver function. In support of this, a copy of Schindl et al., *Gut* 2005 **54(2)**:289-296 is submitted herewith,

Therefore, it is respectfully submitted that one skilled in the art cannot deduce from Nishihara that

administering L-valine improves a condition of low albumin levels, because the rat used in Nishihira would not be in a condition of low albumin level.

Additionally at Sekas et al., page 449, right column, line 3 from the bottom to page 450, left column, line 2. state, "An abrupt decrease in albumin synthesis would not be expected to cause a rapid drop in concentration, since ht half-life of albumin in the circulation is 17-21 days." That is, in the normal state, the amount of albumin newly produced corresponds to that which is reduced, thereby maintaining albumin at a constant level.

However, in the examples of the present application, the balance between production and metabolism in rats was greatly disrupted. The remaining hepatocytes did not have enough function to produce more albumin. For these reasons, the albumin level in the rats rapidly decreased.

As can be seen from Table 1 on page 13 of the present specification, continuous administration albumin for only one day improved the level of albumin. Significant improvement of the albumin level was observed after continuous administration of L-valine for 2 or 4 days (see Tables 1 and 4). If the improvement of the low albumin level is only due to regeneration of the liver, it requires at least one week more to improve the albumin level, considering the half-life

of the albumin. This means that the low albumin level was improved by the action of L-valine rather than the action of regenerating hepatocytes. The method as claimed here has the unexpected effect of rapidly improving the albumin level.

Since Nishihara is directed to a method for liver regeneration, the method presently claimed, namely, a method for treating or improving a low albumin level, particularly hypoalbuminemia, is clearly different from the method of Nishihara. Further, it is respectfully submitted that one skilled in the art would not anticipate this rapid improvement or treatment of a low albumin level having read the Nishihara disclosure.

Claim 35 is rejected under 35 U.S.C. 103(a) as being unpatentable over Kleinberger.

This rejection is respectfully traversed.

As discussed *supra*, hepatic encephalopathy is not the same condition as low albumin levels, and one skilled in the art would not be motivated to treat hepatic malfunction, particularly low albumin levels, by treating hepatic encephalopathy. Kleinberger disclose treating low albumin level by treating hepatic encephalopathy, since there is no relationship between reducing ammonia in tissue and improving the albumin level.

It is assumed that the references cited but not relied upon for rejections are merely cited as being of interest.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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Br. J. exp. Path. (1979) 60, 447

THE EVALUATION OF LIVER FUNCTION AFTER PARTIAL HEPATECTOMY IN THE RAT: SERUM CHANGES

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Summary.—In serial studies of hepatic function in rats after 70% partial hepatectomy, quantitative changes were found in several of the serum components used clinically to assess liver status. The activities of the following enzymes were found to increase: γ-glutamyl transpeptidase and lactic dehydrogenase were maximal 6 h postoperatively, while glutamic oxaloacetic transaminase and alkaline phosphatase reached peak values at 24 and 48 h respectively. Albumin levels were found to be relatively constant during the study; however, total protein concentration was lowest 6–12 h postoperatively, paralleling a decrease in globulin concentration. Bilirubin levels were elevated to 4× normal within 12 h after surgery. After partial hepatectomy calcium and phosphorus concentrations were significantly decreased at 24 and 12 h respectively. With the exception of alkaline phosphatase, the activities of all serum components measured returned to normal levels by 1 week after surgery; the alkaline phosphatase concentration continued to be elevated 2 weeks postoperatively.

A SURVEY of the literature reveals extensive studies of the intracellular changes occurring in the liver after partial hepatectomy in the rat (Ludewig, Minor and Hortenstine, 1939; Sulkin and Gardner, 1948; Bucher and Malt, 1971); however, little information is available on the effect of partial hepatectomy on the serum concentrations of those substances used to assess liver function. In this report we describe the serial quantitative changes in 12 serum components at times from 6 h to 14 days after two-thirds partial hepatectomy.

MATERIALS AND METHODS

Sprague–Dawley male rats weighing 190–210 g were housed in an area with a 12-h light–dark cycle (light 6 a.m.–6 p.m.). The animals were allowed food (Purina Rat Chow, Ralston-Purina Co., Inc., St. Louis, Mo.) and water *ad libitum*. The rats were partially hepatectomized by a standard method (Higgins and Anderson, 1931) under ether anaesthetic. Control animals were subjected to a sham operation in which the liver was gently palpated. All operations were performed between 9 a.m. and 11 a.m.

At 6, 12, 24, 36 and 48 h and at 7 and 14 days after surgery, under light ether anaesthesia the animals were killed by decapitation and mixed arterial–venous blood collected directly. This method of blood collection was compared in control experiments with collection by cardiac puncture; no significant differences in any of the measured serum components were found.

The collected blood was allowed to clot at 4°. The serum was separated by centrifugation using "Sure-Sep" serum–plasma separators (General Diagnostic Co., Morris Plains, N.J.); it was analysed for the following components: albumin, globulin; total protein; total bilirubin; alkaline phosphatase, E.C. 3.1.3.1; lactic dehydrogenase, E.C. 1.1.1.27; γ-glutamyl transpeptidase, E.C. 2.3.2.-; glutamic oxaloacetic transaminase, E.C. 2.6.1.1; total lipids; cholesterol; calcium; and phosphorus. All analyses were performed on a Technicon SMA 12/60 automatic clinical analyser (Technicon Instrument Co., Tarrytown, N.Y.) by Medi-Comp Laboratories Inc. (Cleveland, Ohio) using standard Technicon methodology.

As controls for the alterations in growth rate resulting from partial hepatectomy, additional unoperated animals were included in some of the

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experiments and killed at 1 and 2 weeks with the operated animals. One week after partial hepatectomy the average weight of the partially hepatectomized, sham-operated, and intact rats were 210, 210 and 257 g respectively. After 2 weeks these weights were 250, 293 and 294 g respectively. No differences were found in any of the serum values of the intact rats at the various times and weights described. The results were expressed as the mean ± 1 s.d. Groups ranged from 5 to 11 animals in size, with the exception of the 2 groups killed at 48 h after surgery, which included 4 animals each. Significant differences between the means were determined using Student's t test (Huntsberger, 1967), the significant level being $P < 0{\cdot}05$.

RESULTS AND DISCUSSION

Serum enzymes

The serum enzyme activities after partial hepatectomy are shown in Fig. 1.

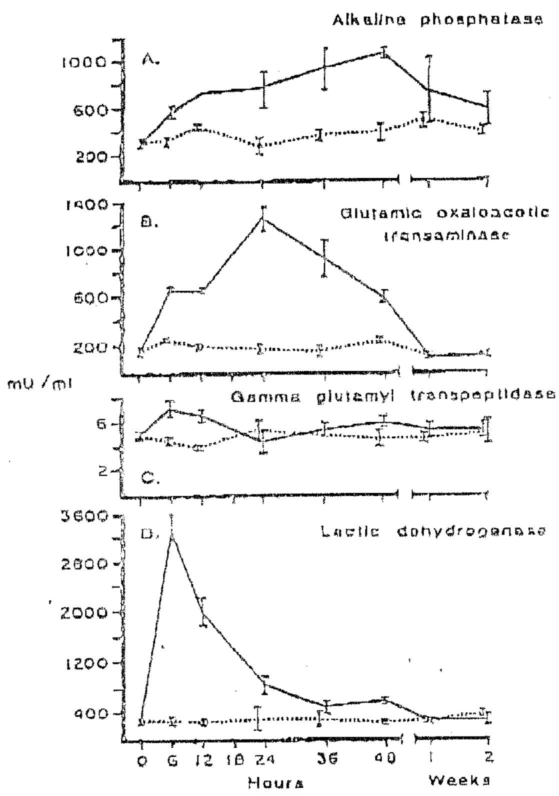

FIG. 1.—Enzyme activities in rat sera at various times after partial hepatectomy; ··· control, —— partially hepatectomized. Bars represent one standard deviation.

γ-Glutamyl transpeptidase and lactic dehydrogenase (Figs 1C, 1D) reach maximum levels 6 h after partial hepatectomy, while alkaline phosphatase and glutamic oxaloacetic transaminase peak at 48 and 24 h respectively (Figs 1A, 1B). γ-Glutamyl transpeptidase levels have returned to normal by 24 h after surgery; lactic dehydrogenase and glutamic oxaloacetic transaminase are normal by 1 week. The decrease in alkaline phosphatase to normal levels is more protracted, the plasma level remaining elevated 2 weeks after operation. Oppenheimer and Flock (1947) have reported that the alkaline phosphatase level is elevated in the plasma after 70% partial hepatectomy; in that study, the highest values occurred 2 days after surgery and decreased gradually to normal in 9 days.

Several mechanisms may account for increases in serum enzyme activities (Zimmerman, 1974); these include enzyme release due to cell necrosis, enzyme release due to increased cell-membrane permeability without cell necrosis, enzyme induction and release, or a decreased disposition of an enzyme.

The clearance of liver enzymes from the circulation has been studied by several groups of investigators. Strandjord, Thomas and White (1959) injected lactic dehydrogenase into dogs and found that the enzyme was cleared in 7–10 h. The normal disappearance curves found in nephrectomized or hepatectomized dogs, in conjunction with data obtained after intraportal injection of enzyme, indicated that the liver and kidney were not responsible for clearing lactic dehydrogenase from the circulation. Frankl and Merritt (1959) reported that i.v. administration of lactic dehydrogenase in dogs was not associated with increased biliary excretion of these enzymes. Dunn, Martins and Reissman (1958) and Wakim and Fleisher (1963*a*) measured the clearance of an i.v. injection of glutamic oxaloacetic transaminase in dogs; 75% of the injected enzyme disappeared from the circulation within 6 h and the remaining 25% was

cleared within 20–72 h. The rapid disappearance phase was found to be due to diffusion of the enzyme into interstitial fluid. Wakim and Fleisher (1963*b*) showed that when zymosan was injected before the enzyme there was a marked acceleration of enzyme clearance, suggesting that glutamic oxaloacetic transaminase is removed by the reticuloendotheilal system. Since a substantial portion of the reticuloendothelial system exists as the Kupffer cells of the liver, the clearance of substances by these cells might be influenced by the reduced number of reticuloendothelial cells resulting from partial hepatectomy.

Possibly enzyme activities are increased as the result of i.p. absorption of enzymes released by necrosis of the small amount of hepatic tissue remaining distal to the site of ligation. Studies of enzyme release after acute toxic liver injury (Rees and Sinha, 1960; Curtis, Moritz and Snodgrass, 1972; Schmidt *et al.*, 1974) have shown that there is a pattern in which the intracellular enzymes are released into the circulation; cytoplasmic enzymes increase in the serum within a few hours, enzymes found in both the cytoplasm and mitochondria increase next, and finally the mitochondrial enzymes appear. The early appearance of cytoplasmic enzymes was suggested (Rees and Sinha, 1960) to be a reflection of cell-membrane injury or permeability change before the onset of frank necrosis. However, inconsistencies in the appearance of intrahepatic enzymes after toxic injury are known (Curtis *et al.*, 1972) and the general complexity of the balance of enzyme release, synthesis and degradation make firm interpretations hazardous.

In the present experiments lactic dehydrogenase, a cytoplasmic enzyme, and γ-glutamyl transpeptidase, which has both cytoplasmic and microsomal locations (Szewczuk, 1966), peak at 6 h after partial hepatectomy; glutamic oxaloacetic transaminase, which is found in both the cytoplasm and the mitochondria, is maximal at 24 h. However, the activity of alkaline phosphatase, a membrane-associated enzyme, is highest 36 h after partial hepatectomy. Kaplan and Righetti (1970) have reported that liver alkaline phosphatase in the serum increases about 2½-fold by 12 h after bile-duct ligation; this enzyme increase was prevented by cycloheximide, suggesting that the increase represented enzyme induction. Although our data (Fig. 1A) show a similar rise in serum alkaline phosphatase, and might be interpreted to indicate some degree of biliary stasis, no stasis was present upon light microscopic examination of histological specimens. Stenger and Confer (1966) have commented on the presence, visible on electron microscopy, of dilated and irregular bile canaliculi in regenerating rat liver, while Mori, Novikoff and Quintana (1975) have described comparable changes in the bile canaliculi and have reported an associated increased canalicular alkaline phosphatase activity by histochemical measurement.

Overall, the enzyme data appear compatible both with some early release of enzyme by damaged cells or cells with altered permeability, and with longer-term effects perhaps involving increased synthesis and release of enzyme.

Protein

The serial changes in serum total protein and globulin concentrations are shown in Figs 2A and 2B respectively. Serum albumin concentration was fairly constant, ranging from 2·5 to 3·6 mg/100 ml, the average concentration being 2·8 mg/100 ml over the 2 weeks of the study. Albumin levels were essentially identical in the sham-operated and partially hepatectomized animals. Roberts and White (1949) reported that after partial hepatectomy albumin levels undergo a transient rise which is followed by an abrupt drop, the lowest concentration occurring 24 h after surgery. We have not observed this decrease in albumin concentration (data not shown). An abrupt decrease in albumin synthesis would not be expected to cause a rapid drop in concentration, since the

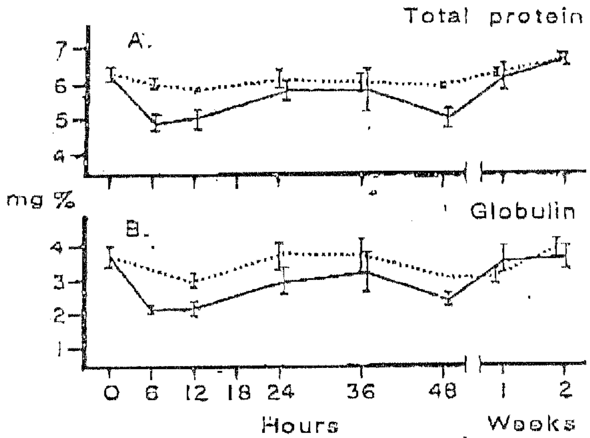

FIG. 2.—Total protein and globulin concentrations in rat sera after partial hepatectomy; ···· control, —— partially hepatectomized.

half-life of albumin in the circulation is 17–21 days (Spiro, 1977).

The decrease in total proteins appears to be primarily due to a decrease in the concentration of globulins. Lowrance and Chanutin (1942) noted that the globulins decrease immediately after partial hepatectomy, and are at their lowest concentration 6–12 h postoperatively; by 36 h after partial hepatectomy this decreased concentration is not statistically significant when compared to the concentrations found in the sham-operated animals. It should be noted that a slight decrease in globulin concentration also occurs immediately after surgery in the sham-operated animals (Fig 2b). Chandler and Snider (1970) have found that after partial hepatectomy there is an increase in the relative rates of synthesis of albumin and the seromucoid fraction of the serum; this increase extends over a period of 14 days after surgery and would tend to mask the early decrease in globulins if only total protein is measured. In their study, laparotomy had no effect on albumin synthesis, but caused an increase in the seromucoid fraction.

Bilirubin

Total bilirubin is increased to a maximum of approximately 4× the normal concentration within 12 h after partial

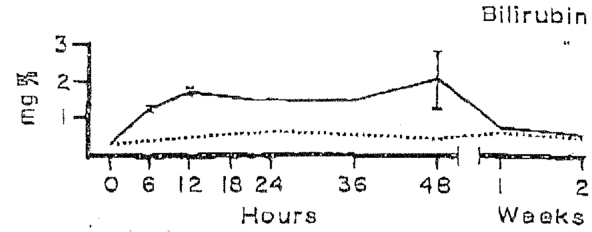

FIG. 3.—Bilirubin concentrations in rat sera after partial hepatectomy; ···· control, —— partially hepatectomized. No standard deviations are shown if the bilirubin concentrations were identical within a group of rats.

hepatectomy (Fig. 3); this increase remains constant for the next 36 h, but by 1 week after surgery the level has returned to normal. Mild cholestasis might account in part for the raised bilirubin (and alkaline phosphatase) levels, but morphological evidence for cholestasis is lacking in our studies, whereas others have described increases in tortuosity and size of bile canaliculi as mentioned above. In functional studies Leong, Pessotti and Brauer (1959) measured bile flow in preparations of regenerating rat-liver remnants isolated at various times after partial hepatectomy. Their data indicated that, at constant perfusion rates, regenerating liver remnants had increased bile flow per unit liver weight up to a maximum increase of 50% by 3 days after operation. During the first 24–48 h the smaller increase they report, coupled with the reduced liver mass, would yield a bile flow considerably below normal, indicating a relative functional deficiency due to loss of liver mass. However, the situation *in vivo* may not be strictly comparable and additional factors may be involved.

Calcium and phosphorus

Rixon and Whitfield (1972) have measured the concentration of plasma calcium at varying times after partial hepatectomy. They reported a decrease in calcium concentration evident at 1 h after surgery, with the concentration reaching the lowest value at 6 h. In our series no determinations of calcium concentration were made before 6 h after partial hepat-

ectomy. We found a statistically significant decrease in calcium concentration to occur in the sera of the partially hepatectomized animals at 24 h; the calcium concentration was 10·5 ± 0·3 mg/100 ml for the sham-operated animals and 8·5 ± 0·6 mg/100 ml for the partially hepatectomized animals. The calcium level returned to normal within 36 h after surgery.

Measurement of the serum phosphorus levels revealed a significant decrease at 12 h after partial hepatectomy; the concentration in the sham-operated animals was 9·1 ± 0·3 mg/100 ml, while the concentration in the partially hepatectomized animals was 7·8 ± 0·4 mg/100 ml. There was no difference in the phosphorus concentration in the 2 groups of rats 24 h postoperatively.

Lipids

Figures 4A and 4B show the concentration of total lipids and cholesterol respectively after partial hepatectomy. Triglyceride and cholesterol ester levels were not determined separately in these experiments. The fluctuation in total lipid concentration parallels that occurring with cholesterol. There is no statistically significant difference in either of the serum components measured between the partially hepatectomized and the sham-operated rats. It is difficult to interpret the changes occurring in the serum lipids in these experiments, although it has been shown (Redgrave, 1970) that ether anaesthesia increases the plasma concentrations of both cholesterol and triglycerides over the short term. One interpretation of the profiles shown would be an immediate increase of lipids after anaesthesia, followed by a gradual return to normal levels, with reduced feeding the night after surgery and increased feeding the night after that. Fisher and Fisher (1963) have reported food intake to be 5% of normal in the first 12 h and about 25% of normal during the second 12-h postoperative period, intakes being increased during the second and third postoperative days. Clearly, the increase of total plasma lipids 4–16 h after feeding can be much greater in normal rats (Dunn, Wilcox and Heinberg, 1975) than the increases (Fig. 4) after *ad libitum* feeding on the second postoperative night.

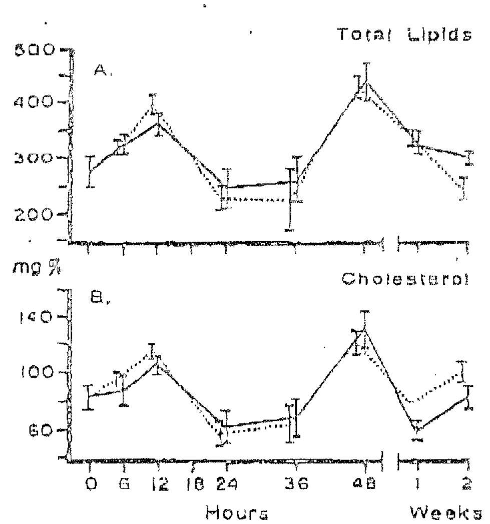

FIG. 4.—Total lipid and cholesterol concentrations in rat sera after partial hepatectomy; ···· control, —— partially hepatectomized.

In conclusion, the present study shows that after partial hepatectomy changes occur in the serum concentrations of several markers used to assess liver function; the serial changes appear to be somewhat different from those which occur during cell necrosis.

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LIVER

The value of residual liver volume as a predictor of hepatic dysfunction and infection after major liver resection

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Background and aims: Major liver resection incurs a risk of postoperative liver dysfunction and infection and there is a lack of objective evidence relating residual liver volume to these complications.
Patients and methods: Liver volumetry was performed on computer models derived from computed tomography (CT) angioportograms of 104 patients with normal synthetic liver function scheduled for liver resection. Relative residual liver volume (%RLV) was calculated as the relation of residual to total functional liver volume and related to postoperative hepatic dysfunction and infection. Receiver operator characteristic curve analysis was undertaken to determine the critical %RLV predicting severe hepatic dysfunction and infection. Univariate analysis and multivariate logistic regression analysis were performed to delineate perioperative predictors of severe hepatic dysfunction and infection.
Results: The incidence of severe hepatic dysfunction and infection following liver resection increased significantly with smaller %RLV. A critical %RLV of 26.6% was identified as associated with severe hepatic dysfunction ($p<0.0001$). Additionally, body mass index (BMI), operating time, and intraoperative blood loss were significant prognostic indicators for severe hepatic dysfunction. It was not possible to predict the individual risk of postoperative infection precisely by %RLV. However, in patients undergoing major liver resection, infection was significantly more common in those who developed postoperative severe hepatic dysfunction compared with those who did not ($p = 0.030$).
Conclusions: The likelihood of severe hepatic dysfunction following liver resection can be predicted by a small %RLV and a high BMI whereas postoperative infection is more related to liver dysfunction than precise residual liver volume. Understanding the relationship between liver volume and synthetic and immune function is the key to improving the safety of major liver resection.

Liver resection of primary and secondary malignancies has becoming increasingly important in recent decades.[1-3] Based on promising survival results and a perioperative mortality rate of <5%, the frontiers of liver surgery are extending continuously towards more major liver resections leaving smaller fractions of residual liver.[4-6] At the same time, a significant increase in postoperative morbidity due to hepatic dysfunction and infectious complications following extended liver resection has been reported, even by very specialised centres.[7-10] The paradigm that at least a third of healthy liver tissue should be left to avoid hepatic failure following resection was developed long ago but few data exist to support this arbitrary value in patients with otherwise healthy livers. The expansion of major liver surgery as a treatment option for various liver tumours has presented new challenges to surgeons and physicians in terms of the assessment and management of postoperative complications, particularly those involving hepatic insufficiency and susceptibility to infection.

The liver contains the largest reserve of fixed tissue macrophages in the body (Kupffer cells) and regulates the synthesis of hepatic proteins responsible for recognition and opsonisation of pathogens as part of the innate immune system.[11] It was shown previously that innate immunity is significantly impaired in acute and chronic liver failure and after liver surgery, suggesting a link between changes in innate immune response and postoperative infection following major liver resection.[12-15] The contribution of the liver to maintain various pathways of innate immunity and its relation to liver volume and global hepatic function after liver resection, have hitherto not been explored.

Measurement of total and partial liver volumes based on computed tomography (CT) and magnetic resonance image analysis has become popular to estimate actual graft size before resection in living related liver transplantation.[16-20] Some studies have addressed the predictive value of residual liver volume regarding liver function and complications after major liver resection.[4,21] However, most of this work has been done in patients with chronic liver disease.[22-26] We have shown previously that preoperative estimation of residual liver volume by CT angioportography (CTAP) image based volumetry provides accurate measures of the actual amount of hepatic parenchyma left after liver resection.[27] The aim of the present study was to use three dimensional hepatic volumetry and virtual resection to define the critical residual liver volume associated with the development of hepatic dysfunction in patients with a healthy liver undergoing liver resection. Furthermore, we wished to establish whether there is a relationship between residual liver volume, liver function, and the incidence of postoperative infection.

PATIENTS AND METHODS

Patients and surgical technique

Volumetric analysis of the liver was performed in patients who were admitted to the Hepatobiliary Unit, Royal Infirmary Edinburgh, for a liver resection and had CTAP done as part of their preoperative assessment. No patient had

Abbreviations: CT, computed tomography; CTAP, CT angioportography; BMI, body mass index; ROC, receiver operator characteristic; TLV, total liver volume; TuV, tumour volume; TFLV, total functional liver volume; RLV, residual liver volume; %RLV, relative residual liver volume

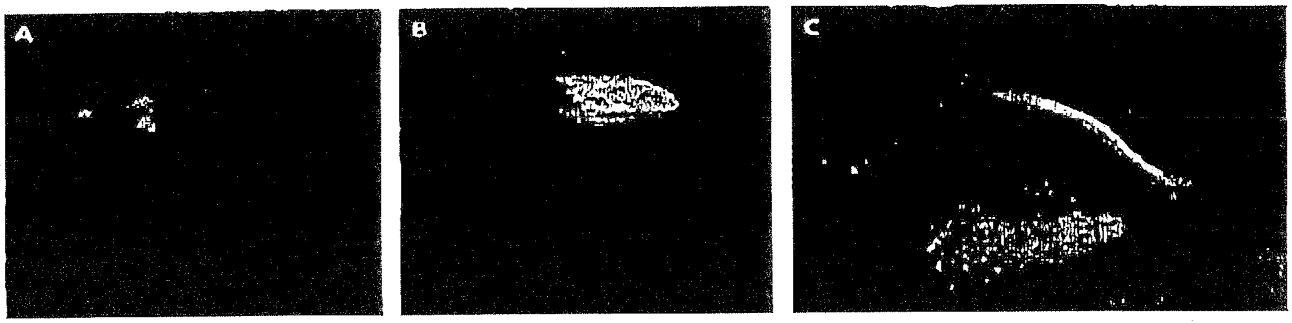


Figure 1 (A–C) Three dimensional liver volumetry of an extended right hepatectomy with caudate excision. (A) Total liver volume (TLV = 1133 ml; red colour); tumour volume (TuV = 34 ml; green colour); and total functional liver volume (TFLV = 1099 ml). (B) Residual liver volume (RLV = 293 ml yellow colour) from three dimensional liver model after virtual resection; relative residual liver volume (%RLV = 26.7%). (C) Intraoperative view of the residual liver following resection.

any background of chronic liver disease and all gave written informed consent. Selection of patients for surgery and the technique of hepatic resection have been described previously.[24] Briefly, surgery for liver tumours was based on segment oriented anatomical resection.[26] With the anaesthetist maintaining a low central venous pressure (3–5 cmH_2O), liver transection was performed using a Cavitron ultrasonic surgical aspirator (CUSA System 200 Macrodissector; Cavitron Surgical Systems, Stamford, Connecticut, USA) without the need for extrahepatic vascular occlusion. The resected liver surface was sealed haemostatically using argon beam coagulation (Force GSU System; Valleylab, Boulder, Colorado, USA). The extent of liver resection was defined according to the number of liver segments removed and grouped into extended (five or more segments), standard (three or four segments), and minor (one or two segments or wedge resection) resections.

Liver volumetry and calculation of relative residual liver volume

Liver volumetry was performed on sets of axial images which were obtained preoperatively during CTAP. The majority of images were taken from a spiral CT GE Hispeed Advantage scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA) using an image slice thickness of 7 mm. In the latest phase of the study, 1 mm image slices were obtained from a multi-slice CT Toshiba Aquilion 16 scanner (Toshiba Medical Systems, California, USA). Medical image analysis software GE Advantage Windows and Analyze 5.0 (Analyze Direct, Inc; Biomedical Imaging Resource, Mayo Foundation, Rochester, USA) were used for volumetry. Every slice from the GE scanner and every fifth slice from the Toshiba scanner were analysed in the following way. The outline of the region of interest was traced manually in each image section, excluding the gall bladder, retro hepatic vena cava, and main branches of the intrahepatic vascular structures. An automated process stacked all slices together to build a virtual model of the liver. Volumetric values were obtained by the inherent software volume rendering algorithm. Total liver volume (TLV) and tumour volume (TuV) were measured and total functional liver volume (TFLV) was calculated by subtracting tumour volume from total liver volume (TFLV = TLV − TuV) (fig 1A). The model of the whole liver was then subjected to virtual hepatic resection according to the operative strategy for each individual patient and the volume of segments to be resected, and residual liver volume (RLV) was measured (fig 1B, C). Relative residual liver volume (%RLV) was expressed as a percentage of TFLV. When the type of resection actually performed was different from that estimated preoperatively, volumetric analysis was repeated.

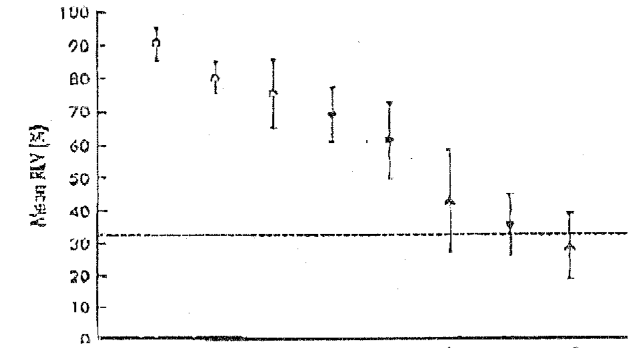
Definition of postoperative complications, hepatic dysfunction, inflammation and infection, and body mass index

Postoperative complications included surgical complications (bleeding from the surgical site and bile leak), hepatic dysfunction, cardiovascular, respiratory, and renal system dysfunction, and infection. These complications were assessed on a daily basis starting from the day of surgery until discharge. Readmission due to problems related to the previous operation was also included. The definition of postoperative hepatic dysfunction was based on serum concentrations of total bilirubin and lactate, prothrombin time, and signs of encephalopathy, categorised into four grades (none/mild/moderate/severe) (table 1).[7 10 11] No, 1, or 2 points were given for each parameter according to the actual results, and summation of all provided the actual score. Parameters were taken into account only when present for two consecutive observations within a 48 hour period. The highest score during the postoperative course determined the severity of hepatic dysfunction (table 1).

The definitions of cardiovascular, respiratory, and renal system dysfunction were taken from two consecutive reports

Table 1 Definition of postoperative hepatic dysfunction based on results from blood tests and clinical observation

Total serum bilirubin (µmol/l)	<20	21–60	>60
Prothrombin time (seconds above normal)	<4	4–6	>6
Serum lactate (mmol/l)	<1.5	1.6–3.5	>3.5
Encephalopathy grade	No	1 and 2	3 and 4
	0	1	2
Severity of hepatic dysfunction	None (0), mild (1–2), moderate (3–4), severe (>4)		



Type of resection	Segment	L Lat Sect	R Post Sect	L Hep	Contr Trisegm	Ext L Hep	R Hep	Ext R Hep
n	6	8	3	5	4	4	48	23
Mean %RLV	90.0	80.1	75.3	68.9	61.1	42.8	35.5	28.7
SD	4.7	4.4	10.3	8.3	11.4	15.6	9.4	10.4

Figure 2 Mean (SD) relative residual liver volume (%RLV) of different types of extended (Ext R Hep, extended right hepatectomy; Ext L Hep, extended left hepatectomy), standard (R Hep, right hepatectomy; Contr Trisegm, central trisegmentectomy; L Hep, left hepatectomy), and minor (R Post Sect, right posterior sectionectomy; L Lat Sect, left lateral sectionectomy; Segment, segmentectomy) liver resection. Reference line indicates 33% RLV.

by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conferences.[12 13] Clinically significant infections only were taken into account and defined as coincidence of positive microbial culture together with either local or general symptoms of inflammation.[13 14] Perioperative death was defined as death within 30 days or during the hospital stay following surgery if this was greater. Severe hepatic dysfunction and infection, but not moderate or mild hepatic dysfunction, had a significant adverse influence on the duration of hospital stay and were chosen as primary end points.

Statistical analysis

Statistical analysis was performed using SPSS 11.0 statistical analysis software (SPSS Inc., Chicago, Illinois, USA). One way between group ANOVA analysis of variance was performed in order to assess differences in %RLV between different types and extent of liver resection and between patients with and without different severities of hepatic dysfunction. The independent sample t test was used to assess differences in %RLV between patients with and without infection. Receiver operator characteristic (ROC) curve analysis was undertaken to identify the value of %RLV in predicting severe hepatic dysfunction and infection with a sensitivity of at least 90% and a specificity of not less than 85%. The positive predictive value of the critical %RLV for severe hepatic dysfunction was calculated for the study group. Univariate analysis of preoperative and intraoperative variables was performed by Pearson χ^2 and Fisher's exact test, respectively, for categorical variables and independent sample t test for continuous variables. Significant variables in univariate analysis were entered simultaneously (forced entry method) into multivariate logistic regression to evaluate their independent predictive value for severe hepatic dysfunction and infection.

RESULTS

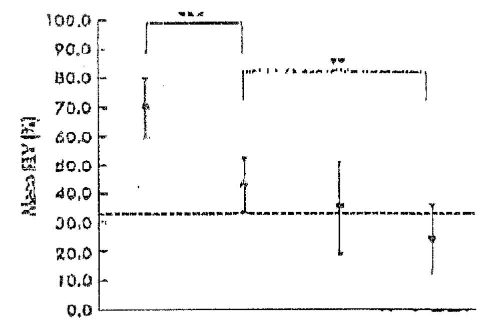
Patient demography, diagnosis, and the extent of liver resection

Volumetry of the liver was performed in 104 patients (59 males and 45 females; mean age 61 (SD 12) years) who consecutively underwent liver resection. The diagnosis was colorectal cancer liver metastasis in 92 (88.5%), other secondary malignancies in six (5.8%), adenoma in two (1.9%), and hepatocellular carcinoma, cholangiocarcinoma, haemangiosarcoma, and focal nodular hyperplasia in one (1.0%) patient each. In total, 27 (26.0%) extended resections, 57 (54.8%) standard resections, and 20 (19.2%) minor resections were performed (fig 2). Mean %RLV was 30.8 (SD 12.1)% after extended liver resection, 40.2 (14.5)% after standard resection, and 82.9 (9.2)% after minor resection (one way between groups ANOVA: p = 0.007 between standard and extended resection; p<0.0001 between minor and standard or extended resection). In 36 of 104 (34.6%) patients, less than 33% RLV was left after resection.

Table 2 Hepatic dysfunction and infection following minor, standard, and extended liver resection

	Extent of liver resection		
	Minor (n = 20)	Standard (n = 57)	Extended (n = 27)
Postoperative hepatic dysfunction***			
No	17 (85.0)	9 (15.8)	1 (3.7)
Mild	3 (15.0)	28 (49.1)	11 (40.7)
Moderate	0	15 (26.3)	7 (25.9)
Severe	0	5 (8.8)	8 (29.6)
Infection***	3 (15.0)	14 (24.6)	16 (59.3)

**p = 0.001, ***p<0.0001 by Pearson χ^2.
Values in parentheses are percentages of patients in each category by extent of liver resection.



Hepatic dysfunction	No	Mild	Moderate	Severe
n	27	42	22	13
Mean %RLV	67.9	42.9	35.3	24.2
SD	22.6	13.4	15.3	6.0

Figure 3 Mean (SD) relative residual liver volume (%RLV) in patients with no, mild, moderate, and severe hepatic dysfunction following liver resection (one way between group ANOVA; **p = 0.005, ***p<0.0001). Reference line indicates 33% RLV.

Postoperative complications and their relation to residual liver volume

In 54 of 104 (51.9%) patients, one or more complications became evident following liver resection. Thirty three (31.7%) patients developed postoperative infection as the most common cause of complications. Mild, moderate, and severe hepatic dysfunction were evident in 42 (40.4%), 22 (21.2%), and 13 (12.5%) patients. Pleural effusions were found in 11 (10.6%), a bile duct leak in six (5.8%), renal dysfunction in four (3.8%), pulmonary embolism in three (2.9%), temporary atrial fibrillation in three (2.9%), bleeding from the surgical site requiring reoperation in two (1.9%), and portal vein

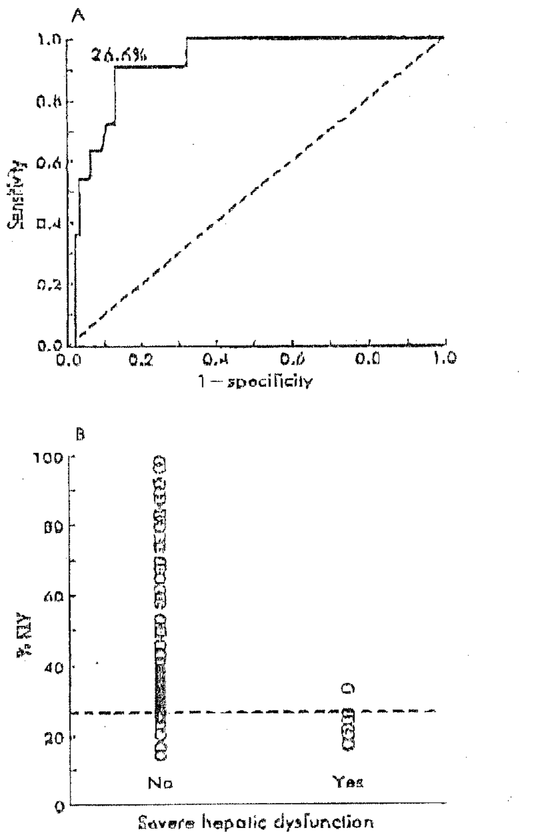


Figure 4 (A) Receiver operator characteristic (ROC) curve analysis of relative residual liver volume (%RLV) to predict postoperative severe hepatic dysfunction. A critical %RLV value of 26.6% was identified (area under the curve = 0.918 (95% confidence interval 0.854–0.983); p<0.0001). (B) Incidence of severe hepatic dysfunction following liver resection according to %RLV. Reference line indicates the critical %RLV of 26.6% associated with a significant risk of postoperative severe hepatic dysfunction (Fisher's exact test; p<0.0001).

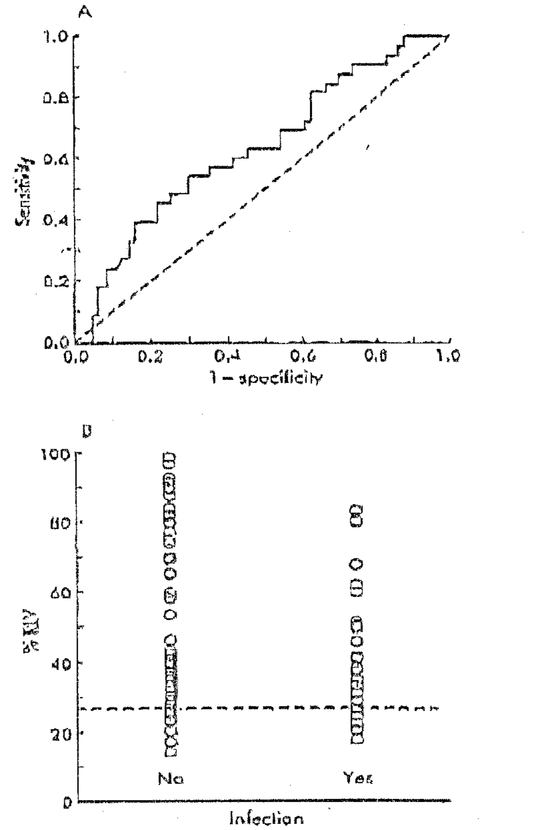


Figure 5 (A) Receiver operator characteristic (ROC) curve analysis of relative residual liver volume (%RLV) to predict postoperative infection. No critical %RLV was identified in predicting infection with precision (area under the curve = 0.641 (95% confidence interval 0.528–0.755); p = 0.021). (B) Incidence of infection following liver resection according to %RLV (Fisher's exact test, p = 0.069). Reference line indicates 26.6% RLV.

thrombosis and upper gastrointestinal tract bleeding in one (1%) patient each. Two of 104 (1.9%) patients died; both developed liver failure (in one associated with sepsis) following extended liver resection. The incidence of postoperative hepatic dysfunction in general and severe hepatic dysfunction in particular increased with the extent of liver resection (table 2). Five of 57 (8.8%) patients after standard liver resection and eight of 27 (29.6%) patients after extended liver resection, but none after minor resection, developed severe hepatic dysfunction.

Mean %RLV was significantly smaller in patients who developed mild hepatic dysfunction compared with those without hepatic dysfunction (42.9 (15.4)% and 69.9 (22.6)%; p<0.0001) (fig 3). Patients who developed severe hepatic dysfunction had a significantly smaller mean %RLV compared with those with mild hepatic dysfunction (24.5 (6.0)% and 42.9 (15.4)%; p = 0.005) (fig 3). The number of patients who developed postoperative infection increased from minor to standard liver resection and was highest after extended liver resection (table 2). Mean %RLV was significantly smaller in patients with postoperative infection compared with those without infection (38.8 (18.5)% and 49.3 (23.6)%; independent sample t test, p = 0.016).

Receiver operator characteristic (ROC) curve analysis of %RLV to predict severe hepatic dysfunction and infection following liver resection

ROC curve analysis revealed that a %RLV value of 26.6% identified patients at significant risk for severe hepatic dysfunction following liver resection (fig 4A). Using this value it was possible to predict severe hepatic dysfunction in patients undergoing liver resection with 90.9% sensitivity and 87.1% specificity. The positive predictive value for the study group was 50.2%, the likelihood ratio for a positive test result (≤26.6% RLV) associated with severe hepatic dysfunction was 7.0, and for a negative test result (>26.6% RLV) 0.1. Ten of 22 (45.5%) patients with a %RLV value of ≤26.6% developed severe hepatic dysfunction compared with one of 82 (1.2%) with a larger %RLV (p<0.0001) (fig 4B). However, 12 of 22 (54.5%) patients with a %RLV value below the critical %RLV did not develop severe hepatic dysfunction. It was not possible to identify a precise %RLV value for predicting postoperative infection by ROC curve analysis (fig 5A). Applying the critical %RLV of 26.6% to predict

Table 3 Preoperative and intraoperative categorical predictors for severe hepatic dysfunction and infection

		Severe hepatic dysfunction	p Value	Infection	p Value
Sex					
Male	59	9 (15.3)	0.109*	22 (37.3)	0.204*
Female	45	2 (4.4)		11 (24.4)	
Extent of resection					
Minor	20	0	0.007**	3 (15.0)	0.001**
Standard	57	4 (7.0)		14 (24.6)	
Extended	27	7 (25.9)		16 (59.3)	
Hepatic inflow occlusion					
No	76	9 (11.8)	1.000*	25 (32.9)	1.000*
Yes	16	2 (12.5)		5 (31.3)	
Blood transfusion					
No	69	3 (4.3)	0.003*	17 (24.6)	0.080*
Yes	25	7 (28.0)		11 (44.0)	

*Fisher's exact test or **Pearson χ^2.

postoperative infection, sensitivity was 33.3% and specificity 84.5%. The likelihood ratio for a positive test result (<26.6% RLV) associated with postoperative infection was 2.1 and for a negative test result (≥26.6% RLV) 0.8. Eleven of 22 (50.0%) patients with a %RLV below 26.6% developed postoperative infection compared with 22 of 82 (26.8%) with a larger %RLV ($p = 0.069$) (fig 5B).

Ability of %RLV in association with other preoperative and intraoperative parameters to predict postoperative severe hepatic dysfunction and infection

Univariate analysis revealed that small %RLV ($p<0.0001$), high body mass index (BMI) ($p<0.0001$), extended liver resection ($p = 0.007$), prolonged operating time ($p = 0.001$), increased blood loss during surgery ($p = 0.007$), and perioperative blood transfusion ($p = 0.003$) were significant predictors of severe hepatic dysfunction following liver resection (tables 3, 4). Extended liver resection ($p = 0.001$), small %RLV ($p = 0.016$), and prolonged operating time ($p = 0.009$) showed significant value in predicting postoperative infection (tables 3, 4). %RLV together with BMI, operating time, intraoperative blood loss, and perioperative blood transfusion were entered into a logistic regression model to identify variables with independent predictive value for severe hepatic dysfunction and infection. A small %RLV and high BMI were found to be significant independent predictors of severe hepatic dysfunction (table 5). However, prolonged operating time and large intraoperative blood loss increased the accuracy of this regression model. By applying BMI, operating time, and intraoperative blood loss to the risk assessment of patients with a small %RLV, it became possible to distinguish between patients who developed severe hepatic dysfunction and those who did not (fig 6). A small %RLV and prolonged operating time were the only two significant independent predictors of postoperative infection (table 5).

Incidence of infection in patients with and without postoperative severe hepatic dysfunction

In order to analyse the relation between impaired liver function and susceptibility to infection, we compared the incidence of postoperative infection between patients who developed postoperative severe hepatic dysfunction and those who did not. The analysis was limited to patients with less than 26.6% RLV because severe hepatic dysfunction was almost exclusively seen in this group. Eight of 11 (72.7%) patients with postoperative severe hepatic dysfunction developed infection whereas this was the case in two of 11 (18.2%) without severe hepatic dysfunction ($p = 0.030$) (table 6).

DISCUSSION

Liver resection is still accompanied by a certain risk of postoperative complications and the overall incidence of complications is significantly increasing towards extended liver resections.[1 4] Hepatic dysfunction and infection are the two most common conditions necessitating prolonged treatment and hospital stay following liver resection.[1 12 13 14] One key to improve the safety of liver resection is to understand the relationship between liver volume and function. There is a lack of evidence to support the assumption that at least one third of a healthy liver should be left to avoid significant hepatic dysfunction, and adequacy of RLV in the past has been based largely on guesswork or crude measures rather than precise measurements. Similarly, the relation between

Table 4 Mean (SD) values of preoperative and intraoperative predictors for severe hepatic dysfunction and infection (independent sample t test)

	Severe hepatic dysfunction			Infection		
	Yes	No	p Value	Yes	No	p Value
Age	59.5 (14.7)	61.1 (11.9)	0.601	63.5 (8.9)	59.8 (13.3)	0.097
BMI	29.9 (6.1)	24.6 (4.2)	<0.0001	26.2 (4.6)	24.6 (4.7)	0.121
%RLV	23.1 (4.9)	48.7 (22.3)	<0.0001	38.8 (18.5)	49.3 (23.6)	0.016
Operating time (min)	264.6 (31.3)	211.3 (53.1)	0.002	237.1 (62.2)	207.5 (52.1)	0.009
Blood loss (ml)	2090.7 (1242.2)	1059.5 (924.2)	0.007	1419.7 (1029.7)	1025.9 (949.4)	0.085

BMI, body mass index; %RLV, relative residual liver volume.

Table 5 Multivariate logistic regression analysis of variables to predict severe hepatic dysfunction and infection following liver resection

	B	SE	Wald	p Value	Exp(B)	95% CI for Exp(B)
Severe hepatic dysfunction						
%RLV	−0.358	0.133	7.271	0.007	0.699	0.539–0.907
BMI	0.280	0.139	4.056	0.044	1.323	1.008–1.738
Blood loss	0.001	0.001	2.194	0.139	1.001	1.000–1.002
Operating time	−0.008	0.013	0.386	0.534	0.992	0.967–1.018
Infection						
%RLV	−0.029	0.010	9.202	0.002	0.971	0.953–0.990
Operating time	0.003	0.002	2.075	0.150	1.003	0.999–1.006

BMI, body mass index; %RLV, relative residual liver volume.

small residual liver, liver dysfunction, and postoperative infection has not been defined.

This study aimed to identify the critical residual liver volume able to predict postoperative severe hepatic dysfunction and to investigate the relationship between small residual liver and postoperative infection. We studied only patients without chronic liver disease to enable an estimate of the maximum capacity of the healthy liver before hepatic dysfunction or infection supervene. An accurate and validated technique of virtual resection in three dimensional liver models was used to calculate the precise volumes associated with liver resection.[27] By subtracting tumour volume from total liver volume, the percentage of RLV in relation to functional liver volume rather than total volume was calculated, thus taking into account the extent of hepatic replacement by large tumours that clearly would not contribute to the functional liver volume. Using this approach, we have demonstrated that volumetric image analysis provides more precise information about the amount of liver tissue left after resection compared with other estimates based on either the type of resection or number of liver segments removed.

There is no consensus on how to define hepatic dysfunction after liver resection and several studies have used different estimates of liver function to describe hepatic functional impairment.[illegible] Some of the variables used to define postoperative hepatic dysfunction, namely alanine aminotransferase, gamma-glutamyl transferase, and alkaline phosphatase, are influenced by the surgical insult to and regeneration of the remaining liver rather than reflecting hepatic function. For the purpose of this study, a novel scoring system of hepatic dysfunction following liver resection was introduced which was derived from routine blood tests and clinical observations. We found a good correlation between liver dysfunction scores and %RLV, and a critical minimum %RLV of 26.6% was identified below which serious hepatic dysfunction is likely to occur. According to our findings, this is the first study using ROC curve analysis to define a precise %RLV that is able to predict the individual likelihood of postoperative severe hepatic dysfunction. Two other studies reported a %RLV of less than 25% as being associated with significant liver dysfunction in patients with normal liver.[illegible] However, the cut off for %RLV in these studies was not derived from precise analysis but rather it was set arbitrarily to compare results of liver function and overall complications between different extents of resection.

We believe that calculating %RLV before major liver resection provides useful information for planning hepatic surgery and in advising individual patients of the potential risks of their surgery. However, it should not be a barrier to undertake major liver resection when the chance for cure and the patient's good condition outweighs the risk. Only half of patients who underwent liver resection leaving less than the critical %RLV developed postoperative severe hepatic dysfunction. However, in all of these patients additional predictive factors, such as high BMI, long operating time, and significant intraoperative blood loss, were evident. BMI was found as a reliable surrogate marker of hepatic steatosis and steatosis is known as a potential risk factor for major liver resection.[illegible] We found a good correlation between the number of liver specimens showing moderate to severe steatosis and BMI, supporting the assumption that steatosis is an important factor for the development of hepatic dysfunction in patients who underwent liver resection leaving only a small %RLV. This observation is of particular

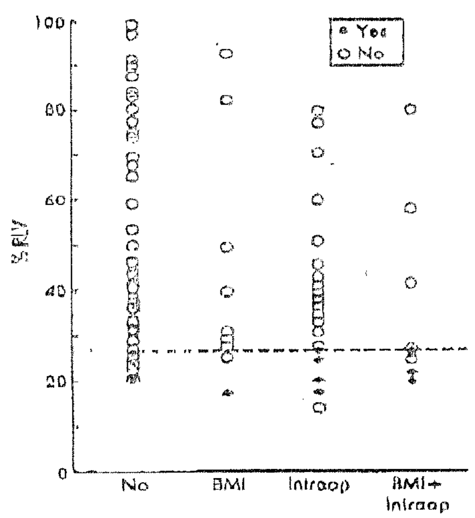


Figure 6 Incidence of severe hepatic dysfunction following liver resection in relation to relative residual liver volume (%RLV) and the presence of the additional risk factors body mass index >30 (BMI), operating time >240 minutes and/or blood loss >2000 ml (Intraop), or both (BMI+Intraop). Reference line indicates the critical %RLV of 26.6%.

Table 6 Relation of severe hepatic dysfunction and infection following major liver resection in patients with a small (<26.6%) residual liver volume

		Severe hepatic dysfunction (%)	
		No	Yes
		11	11
Infection	No	9 (81.8)	3 (27.3)
	Yes	2 (18.2)	8 (72.7)

Fisher's exact test; p = 0.030.

interest as BMI can easily be calculated before surgery and included in the preoperative risk assessment.

Infections are seen frequently after liver resection and in the present study caused a significant proportion of postoperative complications. Several studies suggest an important role for the liver in postoperative innate immune response[11–13] but no single study has investigated the relationship between RLV and the incidence of postoperative infection. Significant loss of hepatic phagocytes (Kupffer cells) together with decreased synthesis of hepatic proteins involved in antigen recognition, opsonisation, and phagocytosis are considered likely to be responsible for the impaired innate immune function following major liver resection and consequently render the patient more susceptible to infection.[11 12 14–16] We found a significant relation between the extent of liver resection, %RLV, and the incidence of postoperative infection. However, a precise %RLV to predict infection with high sensitivity and specificity could not be defined. Postoperative infections are a heterogeneous group of diagnoses.[15] Some may be dependent on the condition of the patient, the extent of liver resection performed, and liver function, but others will be determined by many other factors. Thus lack of a definitive cut off value for %RLV in predicting infection following liver resection might be explained by the study being under powered to account for such heterogeneity rather than there being no true relationship. Analysis of a subgroup of patients with small residual liver volume showed a significant relation between severe hepatic dysfunction and infection, supporting the proposed relationship between liver function, innate immunity, and susceptibility to infection. Studies assessing the reticuloendothelial cell clearance capacity of healthy and diseased liver after major liver resection would be useful in further evaluating the relation between residual liver volume, liver function, and innate immunity.

Estimating %RLV from three dimensional hepatic volumetry and virtual resection in patients undergoing liver resection provides important information in assessing the individual risk for postoperative severe hepatic dysfunction. A critical %RLV of 26.6% was identified below which the risk of developing severe hepatic dysfunction increased significantly. Additionally, obesity renders patients even more likely to develop severe hepatic dysfunction following resection. Although we found an association between small %RLV and postoperative infection, it was not possible to define this risk in terms of a critical %RLV with precision. Studies assessing the various aspects of liver function, including its contribution to innate immunity, and relating these results to actual preoperative and estimated residual liver volume would be of value in understanding the relationship between liver volume and function in further detail. This would also help in developing novel strategies to reduce the incidence of complications related to hepatic dysfunction following major liver resection.

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Conflict of interest: None declared.

APPENDIX

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Gut Tutorial: chronic diarrhoea

Educational objectives

This web based case is designed to take around 30 minutes to complete. You can leave it at any time and return to complete it at another time if you need to. It is aimed at consultant gastroenterologists and specialist registrars in gastroenterology.

After working through this tutorial you should be:

- familiar with the likely causes of painful chronic diarrhoea
- aware of symptom patterns and diagnostic tests for
  - coeliac disease
  - Lactose intolerance
  - Giardiasis
  - Irritable bowel syndrome
  - Crohn's disease
  - Bile salt malabsorption
  - post cholecystectomy diarrhoea
  - Microscopic colitis
  - familiar with management of the above

Clinical details

This 32 year old female school teacher was referred because of longstanding diarrhoea and abdominal pain with recent exacerbation. She had a normal bowel habit, opening her bowels once a day until the age of 26 when she suffered acute bacterial gastroenteritis while on a backpacking holiday in one of the US National Parks. Her main complaint was of colicky lower abdominal pain, which often preceded the urge to defecate.

This pain was usually but not always relieved by defecation. She opened her bowels up to 12 times per day, on awakening and soon after each main meal of the day with occasional bowel movements at other times. She was not often awakened by the need to defecate at night although she did suffer from disturbed sleep. The stool consistency was variable, mainly loose but sometimes normal. She would occasionally pass mucous when the stool was hard. When she had frequent bowel movements there was often anal soreness and streaks of bright red blood on the toilet paper. She also complained of abdominal distension and bloating, worse towards the end of the day. Her appetite was poor and she had lost about 6 lb in weight over the past few months, during which time her symptoms had been worse. She completed a symptom diary which tells the story eloquently.

To take part in this Gut Tutorial, go to http://cpd.bmjjournals.com/cgi/hierarchy/cpd_node;89.